REMARKS

I. The Rejection Under 35 U.S.C. §103(a) Should be Withdrawn

Claims 1-4, 8-11, and 61-62 are rejected under 35 U.S.C. 103(a) as allegedly unpatentable over Marx *et al. Proc Am. Soc. Clin. Oncology* (1997), Vol. 18, No. 1751, p 454 ("Marx") and *Houghton et al. Cancer Chemother Pharmacol* (1995), Vol 36, p 393 ("Houghton). (Office Action, pages 2-3). Applicants respectfully traverse this rejection.

Applicants respectfully submit that the instant claims are not obvious because:

1. The Examiner has not established a *prima facie* case of obviousness because thalidomide was not "well recognized" at the time of the invention for the treatment of cancer "broadly;"

2. There was no reasonable expectation of success at the time of this application in combining topotecan and thalidomide for the treatment of cancer;

3. The art at the time of the invention taught away from the use of thalidomide in cancer therapy; and

4. Sufficient unexpected results are provided to rebut any presumption of obviousness.

Each of these points is discussed below.

1. The Examiner has not established a *prima facie* case of obviousness because thalidomide was not "well recognized" at the time of this application for the treatment of cancer "broadly.

It is alleged in the Office Action that it would have been obvious to combine topotecan and thalidomide for the treatment of cancer. The basis for this allegation is the assertion that "[t]opotecan and thalidomide are well recognized in the art for the treatment of cancer individually." (Office Action, page 4). In reaching his conclusion, the Examiner has relied on the rationale that "use of materials in combination, each of which is known to function for intended purpose, is *prima facie* obvious." (*Id.*, *citing Ex parte Quadrantil*, 25 USPQ2d 1071 (BPAI 1992)). However, Applicants respectfully submit that the Examiner's reliance on this rationale is improperly based on a false premise that thalidomide was "well recognized" at the time of the invention for the treatment of cancer "broadly," as discussed below. (Office Action, page 4).

In order to establish the alleged *prima facie* case of obviousness, the Examiner relies on the proposition that thalidomide was "well recognized" at the time of the invention "to be effective in treating cancer broadly." (*Id.*). In support of this allegation, the Examiner cites to Marx, which discloses results that the authors themselves acknowledge are "preliminary." (Marx, line 21). Moreover, as reported in Marx, when thalidomide was administered to patients having various tumor groups, a partial response to thalidomide was observed in only 2 of the 33 patients assessable for response, and stable disease was observed in less than 1/3 of the treated patients. (Marx, lines 4-9). Since Marx reports that less than 7% of patients exhibited a partial response to thalidomide, it can not be said that thalidomide was "well recognized" to treat cancer on the basis of Marx. Indeed, Applicants respectfully submit that the Examiner has improperly taken Marx out of context to support the alleged *prima face* case of obvious and has disregarded what the teachings of Marx would fairly suggest to one skilled in the art. In this regard, Applicants respectfully remind the Examiner that "it is impermissible to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art." (*In re Wesslau*, 353 F.2d 238, 241, 147 USPQ 391 (CCPA 1965)).

Further, contrary to what is alleged by the Examiner, conflicting assessments regarding thalidomide's efficacy in treating cancer were also well-known in the art at the time of this application. In this regard, Applicants respectfully invite the Examiner's attention to Gutman *et al., Anticancer Research* 16: 3673 (1996) ("Gutman"),[1] entitled "Failure of Thalidomide to Inhibit Tumor Growth and Angiogenesis." As the Examiner will see, Gutman clearly reports that no efficacy was shown when thalidomide was administered to mice having melanoma and colon carcinoma. (Gutman, abstract).

In addition, Applicants also invite the Examiner's attention to Eisen *et al., British Journal of Cancer* (2000), 82(4), 812 ("Eisen").[2] As disclosed in Eisen, 66 human patients having ovarian, renal, melanoma, or breast cancer were administered thalidomide. However, Eisen reports that only 3 of the 18 patients with renal cancer exhibited a partial response to thalidomide. Eisen further reports that none of the 19 patients with ovarian cancer, none of

[1] Attached hereto as **Exhibit A**.
[2] Attached hereto as **Exhibit B**.

the 17 patients with melanoma, and none of the 12 patients with breast cancer exhibited a partial response to thalidomide.

Therefore, Applicants respectfully point out that the teachings of Gutman and Eisen[3] are completely contradictory to the Examiner's allegation that thalidomide was known to be a "well established" agent for the treatment cancer "broadly." Indeed, Gutman and Eisen report that the administration of thalidomide *in vivo* was actually shown to be ineffective in treating certain cancers. Thus, the Examiner's finding of obviousness based on the rationale that "use of materials in combination, each of which is known to function for intended purpose, is *prima facie* obvious" cannot stand where one of the constituents (*i.e.* thalidomide) was not known to function for the intended purpose as instantly claimed (*i.e.* for the treatment of cancer). For these reasons alone, Applicants respectfully submit that no *prima facie* case of obviousness has been established and request that the rejection under 35 U.S.C. §103 be withdrawn.[4]

2. There was no reasonable expectation of success in combining topotecan and thalidomide for the treatment of cancer.

The Examiner has alleged that the combination of thalidomide and topotecan "would have yielded predicable results to one of ordinary skill in the art at the time of the invention" because "Houghton explicitly teaches the treatment of cancer using a topoisomerase I inhibitor (topotecan) in combination with other cytotoxic agents." (Office Action, pages 4-5). Applicants respectfully disagree.

To have a reasonable expectation of success, "one must be motivated to do more than merely 'vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave no indication of which parameters were critical or no direction as to which of many possible choices is likely to be success*ful.*'" *(Medichem, S.A. v. Robaldo, 437 F.3d 1157, 1165* (Fed. Cir. 2006) (*quoting In re O'Farrell*, 853 F.2d 894, 903-04 (Fed. Cir. 1988)). Indeed, "prior art fails to provide the requisite

[3] In addition, Marx, the reference relied on by the Examiner, also appears to be in accord with Gutman and Eisen, as discussed above.

[4] Applicants further submit that reliance on this rationale as a *per se* rule in any obviousness determination is improper, as set forth in Applicants' response of September 7, 2007. As the Examiner is aware, KSR is now the controlling authority with regard to determinations of obviousness under 35 U.S.C. §103. (*KSR International Co. v. Teleflex Inc.* 127 S.Ct. 1727, 167 (L.Ed.2d 705, 75 USLW 4289, 82 USPQ2d 1385)). In this regard, the Supreme Court in KSR expressly warned against the application of "rigid and mandatory formulas" in determining obviousness. (*Id.* at 1741).

'reasonable expectation' of success where it teaches merely to pursue a 'general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.'" (*Id.*, quoting *In re O'Farrell*, 853 F.2d at 903).

In this regard, even while Houghton may disclose the possibility combining topotecan with other cytotoxic agents, Houghton does not teach a combination with any specific cytotoxic agent, much less thalidomide.[5] Moreover, other than incorrectly alleging that thalidomide was known to effectively treat cancer broadly, the Examiner has not pointed to any teaching that would have provided "any direction as to which of many possible choices is likely to be successful." (*Id.*). This is precisely what the courts have held not to be a reasonable expectation of success. (*Id.; O'Farrell*, 853 F.2d at 903-904).

Indeed, absent any specific teaching that would have given one skilled in the art a reason to use thalidomide in combination with topotecan for the treatment of cancer, one skilled in the art would not have found any expectation of success in such a combination. This is especially true where thalidomide's efficacy in treating cancer was not at all established in the art, as discussed above. As stated by the Federal Circuit, "there can be little better evidence negating an expectation of success than actual reports of failure." (*Boehringer Ingelheim Vetmedica, Inc. v. Schering-Plough Corp.*, 320 F.3d 1339, 1354, 65 USPQ2d 1961 (Fed Cir. 2003) (emphasis added)). As such, Applicants respectfully point out that no reasonable expectation of success in the claimed combination for the treatment of cancer would have existed, and thus, respectfully request that the rejection under 35 U.S.C. §103 be withdrawn for this additional reason.

3. The art at the time of the invention taught away from the use of thalidomide in cancer therapy.

Applicants respectfully point out that the claimed invention could not have been obvious because there was ample evidence in the art that would have taught those skilled in the art away from the claimed invention. As well settled, "[a] reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from flowing the path set out in the reference..." (*In re Gurley*, 27 F.3d 551, 553, 31 U.S.P.Q.2d 1130 (Fed. Cir. 1994) (emphasis added)). Indeed, "[a] reference will teach away if it

[5] Furthermore, taking a step back, no evidence of teaching or suggestion that thalidomide is a cytotoxic agent is provided by the Examiner.

suggests that the line of development flowing from the reference's disclosure is unlikely to be productive of the result sought by the applicant." (*Id.*) (emphasis added). Further, in *Yamanouchi Pharmaceutical Co. Ltd. v. Danbury Pharmacal, Inc.*, 231 F.3d 1339, 1344-45, 56 U.S.P.Q.2d 1641 (Fed. Cir. 2000), the Federal Circuit held that the selection of a compound as a lead compound for drug development was not obvious even though the compound exhibited activity that was three times greater than the benchmark compound. In explaining its determination, the Court focused on the disclosure of better alternatives that were up to ten times more active than the benchmark compound and held that the required motivation was not shown. (*Id.*).

As set forth *supra*, Gutman and Eisen reported at the time of this application that thalidomide may be ineffective in the treatment of various cancers *in vivo*. Further, as set forth in Applicants' response of September 7, 2007, the administration of thalidomide to pregnant women was well known to cause birth defects. (Specification, page 6, line 38-page 7, lines 1-5). In view of this information, one skilled in the art would have been discouraged from using thalidomide in the treatment of cancer, much less using it in combination with topotecan in the treatment of cancer. Instead, similar to *Yamanouchi*, one skilled in the art would have selected a "better" alternative to thalidomide – *i.e.* a compound that was known to be effective in treating various cancers, while not causing any adverse effects – to use in combination with topotecan in the treatment of cancer. Thus, under the legal principles set forth above, the art at the time of the invention would have taught those skilled in the art away from using thalidomide in combination with topotecan for the treatment of cancer, and thus, the rejection under 35 U.S.C. §103 should be withdrawn.

4. Sufficient unexpected results are provided to rebut any presumption of obviousness.

Applicants respectfully submit that, even assuming, *arguendo*, that a *prima facie* case of obviousness were established, sufficient unexpected results were provided by Applicants to rebut any presumption of obviousness.

In this regard, Applicants previously submitted an article from the National Cancer Institute, which disclosed that 47% of 30 patients who received thalidomide plus topotecan experienced a complete or partial response, significantly more than the 21% of 39 patients who received topotecan alone. (*See* Exhibit B of Applicants Response of September 7,

2007). Moreover, "[a]dding thalidomide to a topotecan regimen for recurrent ovarian cancer significantly enhances therapeutic response and increases progression-free survival without increasing incidence of toxicity." (*Id.*, emphasis added).

In response, the Examiner alleges that "contrary to applicant's assertion, one of ordinary skill in the art would indeed expect an additive effect in regards to combination therapy." (Office Action, page 5). At the outset, Applicants respectfully point out that there would have been no expectation of an "additive effect" when thalidomide was not known to have an established efficacy in treating cancer, as discussed above.

Moreover, Applicants respectfully point out that, as clearly discussed in the specification, the efficacy of the claimed combination is also based on the observation that combining thalidomide and topotecan did not result in an increase in toxicity. (Specification, page 4, paragraphs 36-39). As is known to one skilled in the art, "combinations of agents are limited by overlapping toxicities, and increases in dose intensity or treatment duration can result in considerable toxicity." (Raza *et al.*, *Leukemia & Lymphoma*, 2006, 47, 3, p 438 ("Raza")[6] (emphasis added)). For example, Applicants respectfully invite the Examiner's attention to Riedel *et al.*, *Lung Cancer* (2006) ("Riedel").[7] As disclosed in Riedel, patients with extensive small-cell lung cancer were administered thalidomide in combination with carboplatin and irinotecan (a topoisomerase inhibitor). However, the treatment was terminated because "[n]o patients were able to tolerate thalidomide beyond ~2 months from study entry. All enrolled patients discontinued thalidomide due to intolerances and associated adverse events." (Riedel, column 1). As such, it is an unexpectedly superior result when agents are successfully used in combination without a significant increase in side effects, especially where, such as here, the two agents are both known to produce serious adverse effects. Such a consideration is absent from the Examiner's reasoning.

The Examiner further alleges that the submitted unexpected results are not commensurate with the scope of the claims because "[t]he instant claims are drawn to the treatment of cancer broadly –*i.e.*, not limited to the treatment of ovarian cancer." (Office Action, page 5).

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[6] Attached hereto as **Exhibit C**.
[7] Attached hereto as **Exhibit D**.

First, Applicants again respectfully invite the Examiner's attention to Raza. As disclosed in Raza, patients with myelodyplastic syndrome ("MDS") were administered topotecan/thalidomide combination therapy. (Raza, abstract). Of the 38 patients evaluated for response, 25% had hematologic improvement and 34% had stable disease. (*Id.*). Notably, "[d]espite predisposing factors, such as the relatively high mean age of the current trial population, very few patients in the current trial experienced any high-grade nonhmeatologic toxicities." (*Id.*, p 439). Indeed, the combination therapy was "generally well tolerated" in patients. (*Id.*).

As is well settled, the nonobviousness of a broader claimed range can be supported by evidence based on unexpected results from testing a narrower range as long as "one of ordinary skill in the art would be able to determine a trend in the exemplified data which would allow the artisan to reasonably extend the probative value thereof." (MPEP §716.02(d)I, *citing In re Kollman*, 595 F.2d 48, 201 USPQ 193 (CCPA 1979)). Thus, based on the efficacy and safety data with regard to ovarian cancer and MDS, one skilled in the art would be "able to ascertain a trend in the exemplified data which would allow him to reasonably extend the probative value" to the entire scope of the claims. (*Id.*). For these reasons alone, Applicants respectfully submit that sufficient unexpected results have been provided to rebut any presumption of obviousness.

Second, as discussed *supra*, the unexpected results are also based, in part, on the observation that combining thalidomide and topotecan did not result in an increase in toxicity. Indeed, Applicants have submitted data showing that thalidomide and topotecan may be safely administered to humans without causing an increase in significant side effects. This observation should then be generally applicable regardless of the specific type of cancer being treated because it is the same two agents that are being administered.

On the other hand, topotecan itself is known to have anti-cancer efficacy against a broad spectrum of cancers. As the Examiner acknowledges, topotecan is "well recognized in the art for the treatment of cancer." (Office Action, page 4). Indeed, as shown in the BCCA Cancer Drug Manual, 2001,[8] Topotecan has been used to treat various cancers including ovarian cancer, small-cell lung cancer, gliomas, actue myelogenuous leukemia, chronic melomonocytic leukemia, non-small cell lung cancer, multiple myeloma, MDS,

[8] Attached hereto as **Exhibit E**.

neuroblastoma, pancreatic cancer, retinoblastoma, rhabdomysarcoma, and Ewing's sarcoma. (*Id.*). Consequently, as the unexpected tolerability of the claimed combination was shown, and such tolerability should be equally applicable to any type of cancers against which topotecan is known to be effective, the unexpected result provided herein is indeed commensurate with the scope of the claims, *i.*e., the treatment of cancer.

For the foregoing reasons, Applicants respectfully submit that sufficient unexpected results, commensurate with the scope of the claims, have been provided to rebut any presumption of obviousness. Thus, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

II. Conclusion

For at least the foregoing reasons, Applicants respectfully submit that all of the pending claims are allowable, and thus, request that the rejections be withdrawn.

A fee of $120.00 is believed due for the Extension of Time. Further, a fee of $810.00 is believed due for the submission of the Request for Continued Examination. If any additional fees are due for the submission of this paper or to avoid abandonment of this application, the Director is authorized to charge them to Deposit Account No. 50-3013.

Respectfully submitted,

Date: March 25, 2008

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Exhibit A

ANTICANCER RESEARCH *16*: 3673-3678 (1996)

Failure of Thalidomide to Inhibit Tumor Growth and Angiogenesis *in Vivo*

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Abstract. *Thalidomide was recently suggested to be an angiogenesis-inhibitor following the demonstration of its activity in a rabbit cornea micropocket model. The purpose of the present study was to test its efficacy in solid tumors in mice. B16-F10 melanoma and CT-26 colon carcinoma cells were injected subcutaneously, intravenously and intraperitoneally, and mice received daily gavage of 0.3-1.0 mg thalidomide starting either two or 10 days following tumor cell injection. The tumors were measured and compared with controls. There was no growth retardation in CT-26 bearing mice nor in mice with pulmonary or peritoneal metastases of B16-F10 melanoma. In 3/7 groups of mice with SC B16-F10 tumors, growth retardation was demonstrated, however the difference was not statistically significant. All tumors eventually reached maximal size, similar to controls. Morphological evaluation of the blood vessels oriented towards the tumor revealed that in both thalidomide and control groups, all mice had developed an intact network of new blood vessels. In our model for the oral administration of thalidomide inhibition of tumor growth and angiogenesis did not occur. We hypothesize that the lack of sustained antiangiogenic response was either due to immune modulation or to tumor heterogeneity and adaptation.*

In order to grow beyond 2 mm in diameter, a tumor must establish a network of blood vessels providing an adequate supply of oxygen and nutrients. This process, known as angiogenesis, has been studied extensively and has been implicated as being an essential step in the pathway of tumor growth and metastasis [1-3]. Moreover, the quantification of the new blood vessels in different human cancers has shown a direct correlation with their potential to invade and produce metastasis [4,5] It is not surprising, therefore, that inhibition of angiogenesis has been the target of novel anticancer strategies [2,6,7]. One agent suggested to be antiangiogenic is thalidomide [8].

Thalidomide was developed in the 1950s as a powerful and safe sedative which appeared to be non-toxic in rodent models. However, soon after its administration in humans it was found to be teratogenic, causing dysmelia (stunted limb growth). Recently, D'amato *et al* [8] postulated that the limb defects seen with thalidomide were secondary to inhibition of vessel buds within the limb. They showed that orally administered thalidomide is an inhibitor of angiogenesis induced by the best known angiogenic molecule, basic fibroblast growth factor (bFGF) in the rabbit cornea micropocket assay [8]. These results suggested clinical applications for using thalidomide in the treatment of diseases involved in pathological angiogenesis, including solid tumors. The purpose of the present study was to study whether thalidomide could inhibit angiogenesis and hence tumor growth in solid tumors *in vivo*.

Materials and Methods

Tumor cell lines. The highly metastatic B16-F10 melanoma and CT-26 colon carcinoma cells were used in this study. B16-F10 cells syngeneic to C57BL mice were a gift of Dr. I.J. Fidler from the M.D. Anderson Cancer Center in Houston, Texas, USA. CT-26, syngeneic to BALB/c mice was a gift from Dr. H. Kashtan of the Tel Aviv Medical Center.

The cells were maintained in Eagle's minimum essential medium,

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Key Words: Angiogenesis, thalidomide, animal models, therapy.

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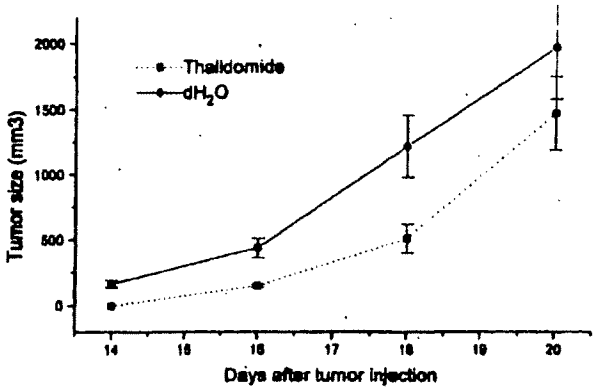

Figure 1. *Growth retardation of B16-F10 melanoma cells injected SC by oral thalidomide administration (300 μg/mouse/day), initiated 10 days after tumor injection.*

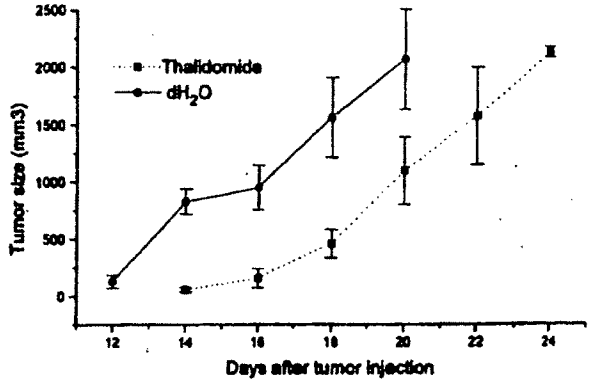

Figure 2. *Growth retardation of B16-F 10 melanoma cells injected SC by oral thalidomide (0.5 mg/mouse/day), initiated two days after tumor injection.*

supplemented with 5 % fetal calf serum (FCS), sodium pyruvate, non-essential amino acids, L-glutamine, a 2-fold vitamin solution (Beit HaEmek Bioproducts, Israel). For *in vivo* studies, tumor cells were harvested from subconfluent cultures by a one minute treatment with 0.25 % trypsin and 0.02 % EDTA. Trypsinization was stopped with medium containing 10% FCS. The cells were washed once and resuspended in phosphate buffered saline (PBS) for injection. Only single cell suspensions with greater than 90% viability (tested by trypan blue exclusion) were used for injections.

Animals and in vivo studies. Female C57BL and BALB/c mice were purchased from the Weizmann Institute of Science (Rehovot, Israel). The mice were maintained in accordance with Israel Ministry of Health and institutional regulations. To produce subcutaneous (SC) tumors, mice were anaesthetized with ketamine; and tumor cells (in 0.1 ml PBS) were injected into the right scapular area. To count the blood vessels tumor cells were injected into the ventral abdominal wall. The resulting SC tumors were measured with a caliper three times a week. Tumor volume was calculated by the formula: volume=$W^2 \times L/2$, where W=short and L=long diameter [9]. To produce pulmonary or intraabdominal metastases, tumor cells (in 0.1 ml PBS) were injected into the lateral tail vein or IP to anesthetized mice. The mice were killed when moribund or at 21 days following tumor cell rejection, autopsied, and the number of metastases counted under a dissecting microscope.

Drugs. Thalidomide, (a gift from Grunentahl GmBH, Germany) was provided as powder. For oral administration in mice, it was suspended in distilled water and prepared daily for each feeding. The suspension was administered to the mice daily by gastric gavage in a volume of 0.1-0.5 ml.

Statistical analysis. The data was analyzed for significance using the student's t-test.

Results

In the first set of experiments, we injected 1 × 10^5 B16-F10 and CT-26 cells to C57BL and BALB/c mice respectively. The cells were injected SC, intravenously (IV) and intraperitoneally (IP) to different groups of mice (n= 10). Ten days later, before any visible tumor could be seen or palpated, each group began to receive a daily gavage of 300 μg/mouse thalidomide in 0.3 ml of dH_2O or dH_2O as control (n=5). Daily feeding continued until termination of the experiment.

In both the IV and IP groups, some animals were moribund after 21-24 days of treatment and were killed by cervical dislocation. At autopsy, both the thalidomide and dH20 groups had multiple (> 50 *per* mouse) metastases. Therefore, no further experiments using IV or IP injections were performed. Fourteen days after injection of B16-F10 cells, visible tumors were noted in 2/5 mice in the SC control group, compared to none in the SC thalidomide group. Thereafter, tumor growth was slower in the thalidomide treated group, reaching a mean volume of 1490 ± 280 mm^3 20 days after injection compared to 1968 ± 390 mm^3 in the control group (Figure 1) (p=N.S.) In the BALB/c group injected SC with CT-26 cells, no difference either in time of tumor appearance or in final tumor size was found between the treated and control groups (2020 ± 512 mm^3 *vs* 1990 ± 482 mm^3 at day 28 after injection, respectively).

Since there were no demonstrable differences within the IP and IV groups, further experiments were performed using SC tumors only. Mice were injected with B16-F10 and CT-26 cells and the daily gavage of 300 μg thalidomide was begun on day two following tumor cell injection, to avoid the possibility that angiogenesis had already begun prior to commencing treatment in the previous set of experiments. In the CT-26 group, no difference could be seen at any time point (data not shown), so all experiments using CT-26 cells were stopped. In the B16-F10 group, there was a marginal difference between the thalidomide-treated mice and controls (1840 ± 411 *vs*

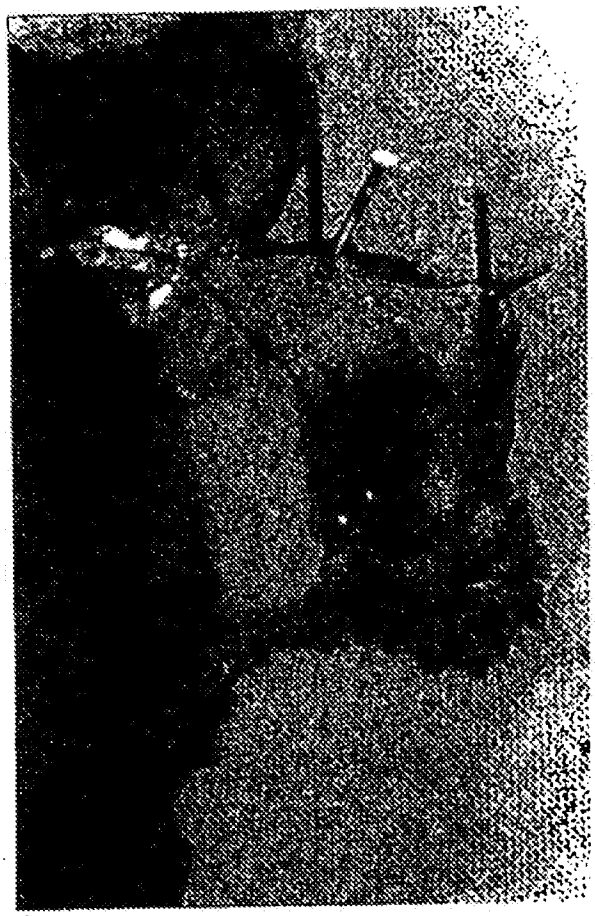

Figure 3. *Macroscopic appearance of B16-F10 tumors growing SC in C57BL mice treated by 0.5 mg/day thalidomide.*

2080 ± 394 mm³, respectively). Therefore, in the next group of mice (n= 10) the thalidomide dosage was increased to 0.5 mg/mouse. The time interval for tumor appearance was shorter in the thalidomide group; at 16 days after tumor cell injection, 2/5 mice had visible tumors compared to 5/5 in the control group. In addition, some delay in tumor growth was noted,although eventually both groups reached the same size (Figure 2). These results, although promising, were not reproducible, and in the next two groups of mice, no difference could be found. To determine whether a higher dose of thalidomide could improve the results, the dosage was increased to 2 mg/mouse in 0.5 ml of dH_2O. No significant difference was found, either regarding time interval for tumor appearance, or eventual tumor size (1704 ± 426 and 2197 ± 510, average of two groups of mice at day 26-28 following tumor cell injection).

In order to perform morphologic evaluation of tumor vascularization, 50,000 B16-F10 cells were injected SC into the ventral abdominal wall of two groups of mice (n=5). Two days later, daily gavage of 0.5 mg thalidomide or dH_2O (control) was begun. On day 12, with tumors measuring 2-5 mm in diameter, the mice were killed. A midline incision was made and the skin dissected from the underlying tissues. The tumor and surrounding skin were photographed and the number of vessels oriented towards the tumor determined by a previously described method [10]. The vessels were readily detectable due to their increased tortuosity and tendency towards loop formation [11]. The average vessel count in both treated and untreated animals was similar (19 ± 3 *vs* 18 ± 4); the thalidomide treated mice had also developed a clearly visible network of new blood vessels (Figure 3).

Discussion

Inhibition of angiogenesis is a novel and exciting modality of cancer therapy. Theoretically it should be non-toxic, since physiologic angiogenesis does not commonly occur in adult life. Angiogenesis can be inhibited by diverse mechanisms. Ezekowitz *et al* have shown that chronic daily administration of low dose interferon (IFN)-α induced complete regression of life-threatening hemangiomas in infants [12]. The effects of IFN-α are likely to be due to downregulation of basic fibroblast growth factor (bFGF) mRNA expression and protein production [13,14]. Thrombospondin (TSP)-1 also inhibits angiogenesis by antagonizing bFGF. TSP-I is regulated by P53, mutation or deletion of which were shown to be associated with loss of TSPI and increased angiogenesis [15]. Recently, a tumor-induced inhibitor of angiogenesis, angiostatin, was introduced, shedding light on some previously unknown mechanisms of tumor progression and metastasis [16].

The understanding of tumor angiogenesis mechanisms has led to many experimental interventions. Systemic administration of antibodies to bFGF [17], vascular endothelial growth factor [18], and angiogenin [19] have all been shown to inhibit the *in vivo* growth of tumor cells. The combination of chemotherapy with angiogenesis inhibitors was synergistic in mice bearing 3LL tumors [6]. Recently, D'Amato *et al* suggested thalidomide to be an angiogenesis inhibitor. Thalidomide is an attractive drug, since it is approved for use in most of the world and, in men and non-pregnant women, is non-toxic. D'Amato *et al* postulated that the limb defects associated with thalidomide were due to the inhibition of angiogenesis and vasculogenesis during fetal development. They tested their hypothesis in a rabbit cornea micropocket assay, and found a correlation between teratogenecity and anti-angiogenic activity. Moreover, electron microscopic analysis of the new blood vessels in the cornea and the deformed limbs showed similar specific ultrastructural changes after treatment with thalidomide [8]. These results inspired the idea of testing thalidomide as an angiogenesis inhibitor in solid tumors *in vivo*.

Although thalidomide is not teratogenic in mice [20], it has immunomodulating and anti-tumor activities when given in doses of up to 100 mg/kg [21].

The present study demonstrates that although thalidomide had no effect at all on the growth of CT-26 colon cancer, there was some trend towards effectivity on the B16-F10 cells growing as SC tumors in mice. Regardless of drug dosage or delivery time schedule, some groups of mice had a longer time interval before tumor appearance, and a slower growth curve. However, tumor growth eventually occurred in all treated animals, and most of the animals tested did not respond to thalidomide. The explanation for the negative results obtained in our experiment may be either host or tumor factors.

The intact, whole animal system is different from the cornea micropocket model the main difference being the extensive involvement of the immune system in solid tumor formation and progression. The relationship between the immune system and tumor angiogenesis is complex. While it is the immune system's duty to eliminate the growing tumor, there is evidence that leukocytes and leukocyte products contribute to angiogenesis, probably by making angiogenic molecules which are utilized by the tumor [9,22]. Thalidomide is an immunomodulator whose best known effect is reducing tumor necrosis factor α levels by enhancing its mRNA degradation [23]. As such, it could be adding to the complex relationship between tumor angiogenesis and the immune system. Administration of thalidomide to tumor bearing mice treated with the anti-tumor agent 5 ,6 dimethylxanthenon-4-acetic acid (5,6 MeXAA) increased the rate of complete tumor regression from 60% to 100% by an as yet unknown mechanism [20].

Tumor factors can also account for the discrepancy between the rabbit cornea and the intact animal. Tumor cells are known to be highly heterogenous [24] and to adapt rapidly to environmental changes [25]. Whilst one pathway of tumor angiogenesis is blocked, the tumor can probably compensate by developing another [3]. This hypothesis could explain the initial delay in tumor growth observed in some B16-F10 bearing mice.

In conclusion, the present study did not demonstrate a sustained, reproducible, anti-angiogenic effect of thalidomide in solid tumors growing in mice. This could be explained as being due either to a compensatory action of host factors such as the immune system, or to tumor heterogeneity and adaptation. The mechanisms which control angiogenesis in different tumors are likely to be highly diverse. Maximizing therapeutic efficacy will require either an individual determination of the "angiogenic profile" of each tumor and the use of a specific anti-angiogenic agent, or a combination of treatment modalities [3].

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Exhibit B

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Continuous low dose Thalidomide: a phase II study in advanced melanoma, renal cell, ovarian and breast cancer

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Summary To grow and metastasize, solid tumours must develop their own blood supply by neo-anglogenesis. Thalidomide inhibits the processing of mRNA encoding peptide molecules including tumour necrosis factor-alpha (TNF-α) and the angiogenic factor vascular endothelial growth factor (VEGF). This study investigated the use of continuous low dose Thalidomide in patients with a variety of advanced malignancies. Sixty-six patients (37 women and 29 men; median age, 48 years; range 33–62 years) with advanced measurable cancer (19 ovarian, 18 renal, 17 melanoma, 12 breast cancer) received Thalidomide 100 mg orally every night until disease progression or unacceptable toxicity was encountered. Three of 18 patients with renal cancer showed partial responses and a further three patients experienced stabilization of their disease for up to 6 months. Although no objective responses were seen in the other tumour types, there were significant improvements in patients' sleeping ($P < 0.05$) and maintained appetite ($P < 0.05$). Serum and urine concentrations of basic fibroblast growth factor (bFGF), TNF-α and VEGF were measured during treatment and higher levels were associated with progressive disease. Thalidomide was well tolerated: Two patients developed WHO Grade 2 peripheral neuropathy and eight patients developed WHO grade 2 lethargy. No patients developed WHO grade 3 or 4 toxicity. Further studies evaluating the use of Thalidomide at higher doses as a single agent for advanced renal cancer and in combination with biochemotherapy regimens are warranted. © 2000 Cancer Research Campaign

Keywords: Thalidomide; TNF-α; renal cell carcinoma

Angiogenesis in melanoma and various carcinomas often correlates with the likelihood of the development of metastases and the prognosis of such patients (Weidner et al, 1991; Folkman, 1995*b*; Weidner, 1995; Erhard et al, 1997; Schiffenbauer et al, 1997). A number of different angiogenic and anti-angiogenic factors are known to be involved in regulation of the angiogenic cascade, including vascular endothelial growth factor (VEGF), acidic and basic fibroblast growth factor (bFGF), hepatocyte growth factor (scatter factor), platelet-derived growth factor, transforming growth factor-β1, interleukin-8 (IL-8), tumour necrosis factor-α (TNF-α), SPARC peptides, angiostatin and interferon-α (Bussolino et al, 1996). These and other cytokines produced by endothelial cells can stimulate tumour cells in a paracrine manner (Hamada et al, 1992). Furthermore, normal endothelial cells within the tumour mass may be abnormally stimulated by diffusible growth factors released by tumour cells (Folkman, 1995*a*).

Angiogenesis inhibitors are now under investigation in patients (Burrows and Thorpe, 1994; Folkman, 1995*b*; Ziegler, 1996). These include the commercially available agents Thalidomide, interferon-α, paclitaxel, tamoxifen and medroxyprogesterone acetate, and experimental agents including neutralizing humanized antibodies to VEGF and bFGF, metalloproteinase inhibitors, TMP-470 (a synthetic analogue of fumagillin) and IL-12 (Ingber et al, 1990; D'Amato et al, 1994; Voest et al, 1995; Gasparini, 1996; Talbot and Brown, 1996). Anti-angiogenic therapy appears to have its optimum efficacy if given daily or intermittently over a long period, acts mainly on proliferating capillary endothelial cells, is generally of low toxicity and, in long-term animal studies, drug resistance has not developed (Folkman, 1995*b*).

Thalidomide is known to have powerful anti-angiogenic activity (D'Amato et al, 1994; Battegay, 1995), can eradicate experimental tumours in mice (Ching et al, 1995) and is used to treat the vascular tumour Kaposi's sarcoma with 60% of patients responding or achieving stable disease (Ziegler, 1996). Non-malignant indications for Thalidomide include retinal neo-vascularization due to macular degeneration, leprosy, Crohn's disease, pulmonary tuberculosis, recurrent graft-versus-host disease and human immuno-deficiency virus-related oral aphthous ulcers (Vogelsang et al, 1992; Asscher, 1994; Crawford, 1994; Folkman, 1995*a*; Tramontana et al, 1995; Jacobson et al, 1997; Wettstein and Meagher, 1997). The notorious teratogenic effects of Thalidomide when administered to women in the first trimester of pregnancy in the 1960s (McBride, 1961; Lenz, 1962) have been attributed to inhibition of blood vessel growth in the developing fetal limb bud (D'Amato et al, 1994) and more recently to free radical-mediated oxidative DNA damage (Parman et al, 1999).

Thalidomide increases the degradation of the mRNA of a number of peptide-signalling molecules such as FGF (D'Amato et al, 1994) and TNF-α (Ching et al, 1995). The suppression of TNF-

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α in cancer patients may be of particular palliative benefit since high levels of TNF-α have previously been linked to cachexia and tumour-related malaise (Yoneda et al, 1991; Haslett, 1998).

The present phase II study assessed the efficacy and toxicity of continuous Thalidomide in the treatment of patients with metastatic melanoma, renal cell carcinoma and ovarian and breast cancer. In view of the dose-related peripheral sensory neuropathy found in patients on long-term Thalidomide, we chose to employ the low dose of Thalidomide 100 mg orally every night in this group of patients, many of whom had previously received neurotoxic chemotherapy, and to follow all patients with electrophysiological studies before and during therapy (Fullerton and Kremer, 1961; Powell and Gardner-Medwin, 1994). Although lower than most doses employed for shorter periods, experience from treating Behçet's syndrome suggests that a Thalidomide dose as low as 25 mg daily is clinically active (Eisenbud et al, 1987). As well as recording the palliative benefits and objective clinical responses to Thalidomide, we assessed biological responses by serially measuring serum TNF-α and serum and urinary VEGF and bFGF before and during treatment.

MATERIALS AND METHODS

Patients

Patients with histologically confirmed metastatic renal cell carcinoma, melanoma, ovarian cancer or breast cancer, were eligible for entry into this study. Eligibility criteria included the presence of metastatic disease that was measurable in at least one diameter greater than 1 cm by clinical examination or imaging. The disease must have shown progression between two recorded time points and be defined clinically or by imaging. Patients must not have received endocrine therapy, radiotherapy, biological agents or chemotherapy in the previous 4 weeks. Marker lesions must not have received radiotherapy at any time. Patients had to be of ECOG performance status 0–2. All patients had to be older than 18 years and had a life expectancy of greater than 12 weeks. Patients required adequate bone marrow reserve with white blood count greater than 3×10^9 l^{-1}, platelets greater than 100×10^9 l^{-1}, and haemoglobin more than 10 g dl^{-1}, normal renal function or a glomerular filtration rate greater than 60 ml min^{-1} as assessed by EDTA clearance and adequate hepatic function defined as liver function tests less than twice the normal values, unless due to metastatic disease. Female patients had to have a negative pregnancy test. Any women of child-bearing age had to use adequate contraceptive methods during the study. In view of the anti-angiogenic action of Thalidomide, any wound must have healed and any surgery other than a skin biopsy must have been performed more than 4 weeks prior to study entry. Patients with pre-existing peripheral neuropathy of WHO grade 2 or greater were not eligible for the study.

The protocol was approved by the Royal Marsden Hospital Ethics Committee and all patients gave written informed consent.

Treatment

Following baseline assessment at study entry, patients started taking Thalidomide 100 mg (Penn Pharmaceuticals Ltd, Gwent, UK) orally every night. Treatment continued until there was evidence of disease progression or unacceptable toxicity was encountered, or the patient wished to stop treatment for any reason.

Assessment of response and toxicity

All patients were examined clinically before treatment. Baseline laboratory investigations included full blood count, serum biochemistry, sensory nerve action potential (SNAP), serum and urine were frozen and stored for bFGF, VEGF and TNF-α assessment by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, UK), electrocardiogram and serum pregnancy test for women of child-bearing age. Patients underwent imaging or clinical measurement as appropriate to assess their disease status at the start of treatment. Patients completed a symptom distress scale (HAD and Rotterdam scores) before starting treatment.

Patients had a clinical examination, full blood count and biochemistry every month whilst on treatment. Patients received a SNAP test, serum and urine were frozen and stored for bFGF, VEGF and TNF-α assessment, clinical imaging, symptom distress scale questionnaire and toxicity evaluation at 1, 3, 6, 9 and 12 months while on treatment.

Response was assessed according to standard International Union Against Cancer criteria following clinical measurement or imaging. Toxicity was assessed according to WHO criteria at each assessment visit (Millar et al, 1981). Patients who received at least 4 weeks of treatment were assessable for response and all patients were assessable for toxicity. The response duration was defined as the time elapsed between start of treatment with Thalidomide and the date of progressive disease or last follow-up evaluation. Stable disease was defined as where neither partial response nor progressive disease could be established.

Statistical considerations

The threshold response rate for each individual tumour type below which the treatment would be considered ineffective was 15%. It was originally intended to recruit 19 patients for each tumour type. If there were no responses in these patients the study would be terminated. There was only a 5% chance that this would occur if the true response rate was 15%. We decided to terminate the study before full recruitment had been completed because of the responses seen and because of slow recruitment of patients with breast cancer. The χ^2 test and McNemar test for trend were used to assess differences in quality of life before and during treatment with Thalidomide.

RESULTS

Patient characteristics

Between July 1997 and March 1998, 66 eligible patients with advanced solid tumours under the care of the Royal Marsden Hospital, London and Sutton, were entered into the study; 19 patients had ovarian cancer, 13 men and five women had renal carcinoma, 12 men and five women had melanoma and 12 women had breast cancer (Table 1). Two other patients with melanoma were also entered but were found not to be eligible because, on review of radiological imaging, they showed a late response to biochemotherapy given prior to entering the study. The median age of patients was 48 years, range 33–62 years. The majority of patients were fit, with ten having a performance status of 0 and 38 having a performance status of 1. However, 18 patients had a performance status of 2. Many of the patients had received multiple treatments before entering the study.

Table 1 Patient characteristics

Number of patients	66
Ovary	19
Renal	18
Melanoma	17
Breast	12
Male	29
Female	37
Age (years)	
Median	48
Range	(33–62)
Performance status	
0	10
1	38
2	18
Previous treatment	
Chemotherapy	44
Radiotherapy	24
Surgery	22
Hormone	14
Immuno-chemotherapy	10
Immunotherapy	4
None	10

Tumour response

All 66 eligible patients entered into the study were evaluable for response and toxicity (Table 2). Three partial responses were seen in the 18 patients treated for renal carcinoma. One response lasted 5 months, the other two partial responses are continuing at 5 and 11 months of follow-up. The response continuing at 11 months was dramatic and is illustrated in Figure 1. This patient had progressed rapidly on biochemotherapy with neck lymphadenopathy and symptomatic lung metastases and had severe malaise before starting Thalidomide. Within 24 h of starting Thalidomide his malaise had resolved and his performance status had improved to 0. Over the next 5 months his lung and lymph node metastases shrunk and a bronchoscopic biopsy of the largest lung metastasis at 11 months showed necrotic tumour only. It was also noticeable in the group with renal carcinoma that 13 patients experienced stabilization of their previously progressive disease: three of these patients had stable disease for 3 months or longer, whilst the other ten patients had stable disease for only 1–3 months.

Of the remaining 48 patients with melanoma, ovarian carcinoma or breast carcinoma none showed any objective tumour response to treatment with Thalidomide. However, three patients had a differential response and one patient with rapidly progressive skin deposits of melanoma on his leg experienced symptomatic improvement and was able to walk more than 100 yards, having previously been chair-bound.

Table 2 Responses to Thalidomide

	No. of patients	Partial response	Stable disease (> 3 months)
Ovary	19	0 (0%)	1 (5%)
Renal	18	3 (17%)	3 (17%)
Melanoma	17	0 (0%)	1 (6%)
Breast	12	0 (0%)	0 (0%)

Table 3 Concentration of angiogenic markers in serum and urine before and during Thalidomide therapy (pg ml^{-1})

Serum	Controls $n = 2$		Pre-Thalidomide $n = 41$		On Thalidomide (4–6 weeks of treatment) $n = 23$	
	Mean	Range	Mean	Range	Mean	Range
VEGF	51	27–74	568	114–1861	554	110–1080
bFGF	< 10	< 10	< 10	< 10–28	< 10	< 10–20
TNF-α	< 10	< 10	< 10	< 10–16	< 10	< 10–20
Urine	Controls $n = 2$		Pre-Thalidomide $n = 31$		On Thalidomide (4–6 weeks of treatment) $n = 13$	
	Mean	Range	Mean	Range	Mean	Range
VEGF	83	30–145	106	24–695	222	10–1000
bFGF	< 10	< 10	< 10	< 10	< 10	< 10
TNF-α	< 10	< 10	< 10	< 10	< 10	< 10

Angiogenic markers

A wide range of serum and urinary VEGF levels was found in patients and in two controls (Table 3). No clear relationship between the absolute level of VEGF and tumour response was found, nor were there any clear differences between different tumour types. However, a rising VEGF level was associated with progressive disease in six of 11 patients who had serial measurements.

bFGF was only detected in the serum of 12 patients all of whom had early progressive disease. bFGF and free TNF-α were not detected in the urine of any patient. Free TNF-α was detected in the serum of one patient with melanoma, one patient with breast cancer and one patient with ovarian cancer.

Toxicity and symptomatic side-effects

Thalidomide 100 mg orally every night was generally very well tolerated with no WHO grade 3 or 4 toxicities (Table 4). The main toxicity was lethargy (38 patients grade 1 and eight patients grade 2). Grade 2 peripheral neuropathy was detected clinically in two patients who had been on treatment for 11 months. On interrupting Thalidomide, the neuropathy resolved and treatment recommenced at 50 mg each night. All patients had SNAP tests and no other patient developed sensory neuropathy. One patient developed a WHO grade 2 headache shortly after starting Thalidomide and another developed WHO grade 2 peripheral oedema. Both side-effects resolved on stopping Thalidomide. Ten patients developed WHO grade 1 constipation and two developed an itchy WHO grade 1 skin rash. These side-effects responded to standard measures.

Table 4 Symptomatic side-effects

	WHO grade 1		WHO grade 2	
	n	(%)	n	(%)
Lethargy	38	58	8	12
Peripheral neuropathy	0		2	3
Headache	0		1	2
Oedema	0		1	2
Constipation	10	15	0	
Skin rash	2	3	0	

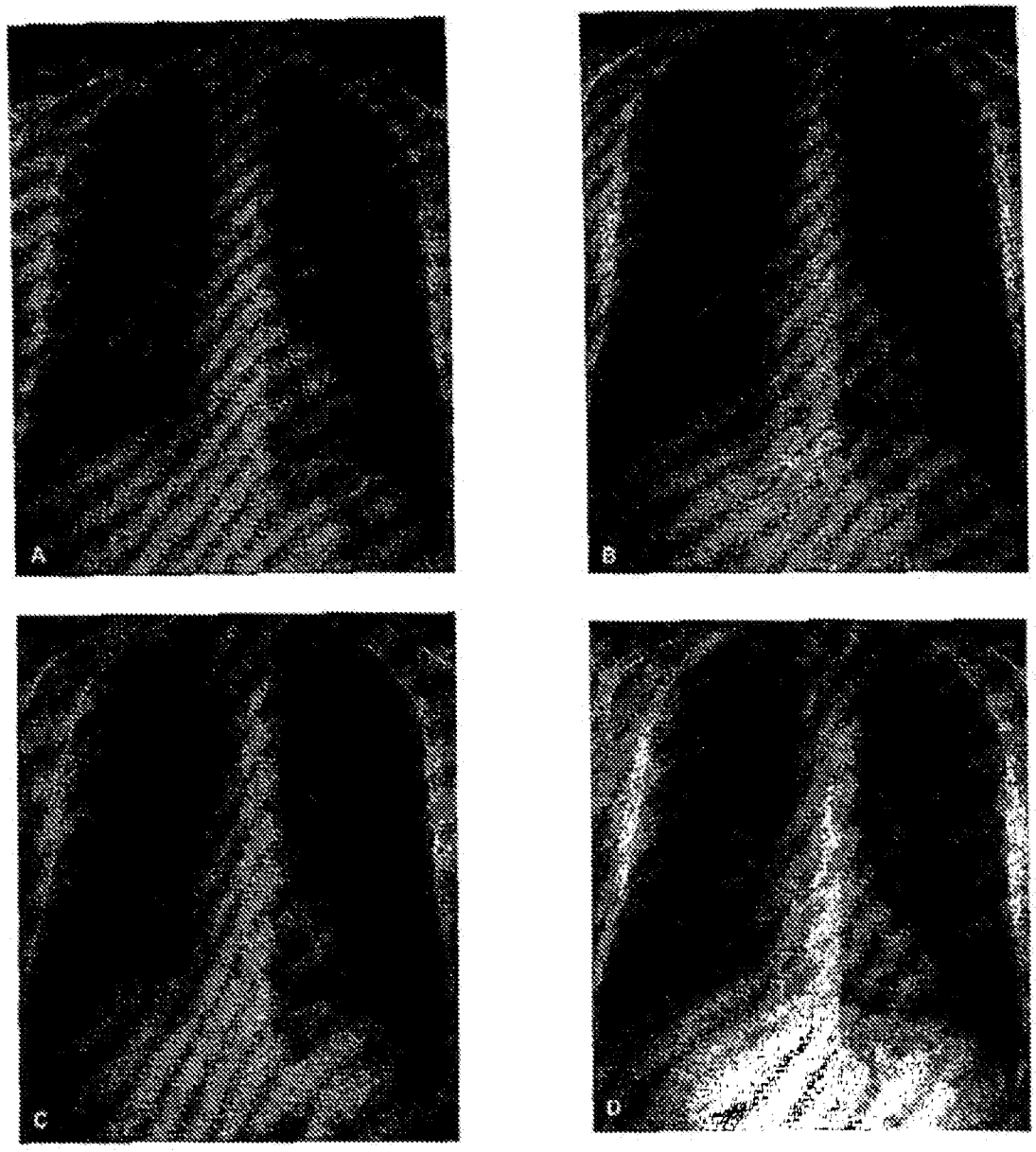

Figure 1 Chest X-ray series illustrating response to Thalidomide after rapid progression on biochemotherapy. (A) At end of biochemotherapy. (B) At start of Thalidomide 6 weeks later, showing progressive disease. (C) After 2 months of Thalidomide showing PR. (D) After 4 months of Thalidomide showing further PR

Of 15 patients who completed serial HAD and Rotterdam scores, 11 experienced an improvement in sleeping and 14 experienced a maintained or improved appetite. Both of these findings are statistically significant ($P < 0.05$), although based on a very small number of patients.

DISCUSSION

Despite employing a low dose of Thalidomide, we observed encouraging responses in patients with renal cell carcinoma. In 18 patients treated for renal carcinoma, three showed partial responses to Thalidomide and 13 showed stable disease, three of them for more than 3 months. Two of the three responding patients had progressed on previous biochemotherapy with interferon-α, 5-fluorouracil and IL-2. Late responses to immunotherapy are seen both in renal cell carcinoma and in melanoma. However, the partial responses in these two patients are unlikely to be due to a late response to biochemotherapy for three reasons. First, patients were found to have rapidly progressive disease both during and after biochemotherapy with disease appearing in previously

unaffected sites. Secondly, the patients obtained significant palliative benefit within 24 h of starting Thalidomide and thirdly, objective tumour shrinkage started within 2 weeks of starting Thalidomide.

We also observed stable disease in 13 of the remaining 15 patients with renal cell carcinoma. In three of these patients the stable disease was observed for more than 3 months. These findings may be important as the patients had aggressive and advanced disease. Four patients with melanoma had stable disease for periods of up to 5 months and one patient with melanoma experienced significant symptomatic relief from his melanomatous leg deposits. However, no objective responses were observed in any of the other patients. Patients found repeated quality of life assessments irksome and this accounts for the poor uptake. The significant symptomatic benefits, including improved sleep and appetite, could be a result of self-selection by those completing the questionnaires. However, the palliative benefits were credible and indeed possibly predictable in view of the known sedative and appetite-enhancing effects of Thalidomide (Powell and Gardner-Medwin, 1994).

Thalidomide could act either by inhibiting angiogenesis or by inhibiting factors secreted by the tumour. A humoural basis for the action of Thalidomide is suggested by the rapid onset of benefit in our responding patients. The most likely candidate target in renal cell carcinoma is TNF-α. This cytokine is known to be secreted by renal cell carcinomas (Mizutani et al, 1994), enhances neo-angiogenesis (Fajardo et al, 1992), augments the stimulation of renal carcinoma cells by IL-6 (Koo et al, 1992) and contributes to many of the systemic symptoms of advanced malignancy (Yoneda et al., 1991; Haslett, 1998). The inhibition of TNF-α by Thalidomide may therefore be of particular interest in renal cell carcinoma.

No clear effect of Thalidomide was observed in serial measurements of serum and urine TNF-α, VEGF and bFGF. Free TNF-α was only detected in the serum of three patients at the time that their progressive disease was noted. Serum TNF-α levels may therefore be of limited value in the selection of patients for Thalidomide treatment or the monitoring of their response. In future studies we will monitor concentrations of TNF-α in fresh plasma samples which may be more reliable. The significance of systemic levels of TNF-α in renal cell carcinoma requires further investigation. It is possible that local concentrations in the tumour bed are of greater significance. We found a wide range of serum and urinary levels of VEGF both in patients and in two controls. In 11 patients who had serial concentrations measured, a rising VEGF level was found in all six patients with progressive disease. Similarly, bFGF was only detected in the serum of 12 patients, all of whom progressed early.

Thalidomide 100 mg orally each night was well tolerated for at least 11 months even in patients who have previously been treated with neurotoxic chemotherapy. Two patients who have received Thalidomide for 11 months and 5 months have developed grade 2 peripheral neuropathy which resolved on interrupting treatment. Many patients noticed mild lethargy and constipation but none developed grade 3 or 4 toxicities.

Our findings suggest that Thalidomide may be useful in the management of advanced renal carcinoma and possibly of symptomatic benefit in other solid malignancies. This warrants further studies, particularly at a higher dose in patients with renal carcinoma. A fuller appreciation of the effect of Thalidomide on TNF-α levels is needed because Thalidomide may be of particular benefit to patients who express high levels of TNF-α. Such a correlation would allow better selection of patients who may benefit from this and other anti-angiogenic therapies. Finally, the use of Thalidomide in combination with other treatments should be investigated. Highest response rates in renal cell carcinoma are reported with a combination of interferon-α, 5-fluorouracil and IL-2 (Lopez-Hanninen et al, 1996). The considerable side-effects may be related to the finding that IL-2 induces high levels of TNF-α (Weidmann et al, 1992). The increased levels of TNF-α are unlikely to contribute to the beneficial effects of this treatment, since previous experience with TNF-α in renal carcinoma has shown severe side-effects with a poor response rate (reviewed by Boshoff and Jones, 1996). Moreover, these high levels of TNF-α may also stimulate growth of renal carcinoma cells (Lopez-Hanninen et al, 1996). Interestingly, two of the three patients who had partial responses to Thalidomide had progressed rapidly on biochemotherapy for their advanced renal carcinoma. It is tempting to speculate that these patients' tumours were responsive to stimulation with TNF-α and therefore grew rapidly on biochemotherapy and responded to inhibition of TNF-α by Thalidomide. There may be a rationale for combining Thalidomide with biochemotherapy, as a possible way both of reducing side-effects and increasing the efficacy of treatment for renal cell carcinoma.

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Exhibit C

Leukemia & Lymphoma, March 2006; 47(3): 433–440

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ORIGINAL ARTICLE: CLINICAL

Phase II study of topotecan and thalidomide in patients with high-risk myelodysplastic syndromes

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Abstract

This phase II trial investigated the safety and preliminary efficacy of a topotecan/thalidomide combination therapy in patients with myelodysplastic syndrome who had refractory anemia with excess blasts (RAEB), RAEB with transformation, or chronic myelomonocytic anemia. Patients received three 21-day cycles of topotecan 1.25 mg/m^2 on days 1–5, which was repeated for two additional cycles in patients whose bone marrow blast percentages did not decrease. Oral thalidomide was then started at 100 mg/day (with the dose escalated up to 300 mg/day if well tolerated) for up to 1 year. Patients were monitored throughout the trial for hematologic and clinical adverse events, and efficacy was assessed using International Working Group (IWG) criteria. Forty-five patients, mostly elderly (median age 68 years; range 52–79 years), were enrolled. Therapy was generally well tolerated compared to high-dose chemotherapy. Three patients died from disease progression/infections during topotecan therapy, and four patients discontinued topotecan because of high-grade neutropenia (two patients), syncope (one patient), or hip surgery (one patient). Of 24 patients who received thalidomide, three discontinued because of treatment-related toxicity. Thirty-eight patients were evaluable for response: nine (24%) had hematologic improvement and 13 (34%) had stable disease. Responses occurred in patients with all disease subtypes. Six patients achieved transfusion independence, and one patient had a trilineage response. Approximately one-third of the patients had decreases in bone marrow blasts of $\geq 50\%$. Therefore, a topotecan and thalidomide combination therapy is promising, although further studies are needed to determine the optimum doses and schedule.

Keywords: *Induction therapy, myelodysplastic syndrome, phase II trial, thalidomide, topotecan*

Introduction

Myelodysplastic syndrome (MDS) is a heterogeneous genre of disorders resulting from the monoclonal expansion of hematopoietic stem cells, and the incidence of MDS is expected to increase with the ageing population in the USA and Europe [1]. The clinical implications of MDS range from mild cytopenias to highly aggressive acute myeloid leukemia (AML). Patients with $\geq 5\%$ blasts in their bone marrow have a relatively high risk of death from complications associated with transformation to acute leukemia, and $>20\%$ blasts in the bone marrow is now considered sufficient for a diagnosis of AML [2]. More than 30% of patients diagnosed with MDS fall into the high-risk category [3]. Even with state-of-the-art care, high-risk MDS is associated with a relatively poor prognosis.

High-dose chemotherapy regimens for MDS are limited by tolerability, and stem cell transplant regimens are limited by the availability of matched donors and by the tolerability of these regimens in elderly patients. Therefore, two-step therapy regimens that combine induction therapy with a cytotoxic agent, such as an anthracycline (e.g. doxorubicin or epirubicin) with cytosine arabinoside (cytarabine; ara-C), are commonly used to treat patients with higher-grade MDS or AML [4]. This approach has been relatively successful compared to single-agent therapies. However, approximately one-half of high-risk MDS patients treated with combination regimens do not respond to therapy, and median overall survival is in the range 3–19 months [5].

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Attempts to increase treatment efficacy by dose escalation have resulted in severe toxicity. Furthermore, the cumulative effects of ara-C can result in prolonged aplasia [6]. Therefore, treatment of patients with high-risk MDS represents an unmet medical need.

Topotecan (Hycamtin®, GlaxoSmithKline, Philadelphia, PA, USA) is currently approved for the second-line therapy of ovarian cancer and small cell lung cancer. Topotecan has also demonstrated intriguing clinical activity in patients with hematologic malignancies [7]. Topotecan binds to DNA topoisomerase I, resulting in DNA breaks and apoptosis, and this mechanism of action is different from that of other agents used in this setting, making it an attractive therapeutic option because of the decreased likelihood of tumor cross-resistance [8]. This agent has been successfully used as single-agent therapy and in combination regimens in the MDS and AML settings [9–15]. A high-dose regimen of topotecan in combination with ara-C was found to be particularly effective and well tolerated in patients with refractory anemia with excess blasts (RAEB) [16]. Topotecan has a manageable hematologic toxicity profile, characterized by noncumulative and reversible neutropenia, as well as a favourable nonhematologic toxicity profile. The safety profile of topotecan compares favourably with that of other DNA-targeting agents used for MDS. Moreover, the noncumulative and reversible nature of its hematologic toxicity makes topotecan well suited for induction therapy, allowing recovery of normal precursor cells before subsequent treatment is initiated.

Thalidomide has demonstrated antileukemic activities in patients with MDS or AML, including effects on cell differentiation and survival and significant reductions in microvessel density in the bone marrow [17–19], which may be an important mechanism to prevent disease progression [19]. This agent has generally low toxicity compared to other antileukemic therapies and produces high response rates in some patient subsets [18,19]. Moreover, thalidomide can be administered orally and is safe for long-term use [18]. These characteristics make it well suited for use as a maintenance therapy in patients with high-risk MDS.

Based on the relative success of combination chemotherapy in patients with high-risk MDS and the demonstrated clinical activity of topotecan and thalidomide in this setting, there is a solid rationale for investigating topotecan/thalidomide combination therapy. Combining these two agents, which have different mechanisms of action, may produce activity similar to HDC but with lower toxicity. Additionally, the use of thalidomide may allow long-term maintenance therapy, which may be necessary to overcome the poor prognosis of high-risk MDS [5]. Therefore, the present study investigated the tolerability and activity of topotecan induction therapy followed by long-term treatment with oral thalidomide in patients with high-risk MDS.

Patients and methods

Eligibility

Adult patients (>21 years of age) with newly diagnosed or previously treated RAEB, RAEB with transformation (RAEB-T), or chronic myelomonocytic leukemia (CMML) were eligible for the study. Eligible patients had an Eastern Cooperative Oncology Group performance status of ≤ 2, adequate renal function (blood urea nitrogen <21 mg/dl; serum creatinine <1.3 mg/dl) and liver function [alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels $\leq 3 \times$ upper limit of normal (ULN)], and a life expectancy >6 months. Patients were excluded from the trial if they had a history of hypertension that required medication, if they had a history of neuropathy, or if they were pregnant or nursing or had active infections that were not effectively controlled with antimicrobial or antiviral therapy. All patients receiving thalidomide were required to practice birth control. The Institutional Review Boards approved the study, and all patients provided their written informed consent.

Study design and treatment

This was an open-label, single-arm pilot study. Patients received topotecan 1.25 mg/m^2 by 30-min intravenous infusion on days 1–5 of a 21-day cycle for three cycles (Figure 1). At the end of this treatment, patients with $\geq 50\%$ decrease in bone marrow blasts or with blasts <5% commenced therapy with thalidomide. Patients with <50%

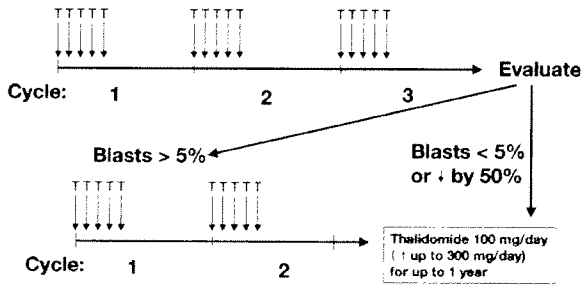

Figure 1. Schematic of trial design. Patients were treated with three 21-day cycles of topotecan 1.25 mg/m^2 (T) on days 1–5. A bone marrow biopsy was then performed. Patients with <5% blasts or with $\geq 50\%$ decreases in blasts immediately began therapy with daily oral thalidomide. Patients with >5% blasts received two more cycles of T before commencing thalidomide.

decrease in bone marrow blasts received an additional two cycles of topotecan before commencing thalidomide. All thalidomide was dispensed according to the guidelines of the System for Thalidomide Education and Prescribing Safety program guidelines [20,21]. Thalidomide was initiated at 100 mg/day orally at bedtime. The dosage was increased as tolerated up to a maximum of 300 mg/day. During treatment with thalidomide, patients received oral supplements including 100 mg/day vitamin B_6 and standard senna concentrate (Senokot®, Purdue Pharmaceuticals, Georgetown, NC, USA) bulk laxative tablets. Thalidomide was continued for up to 1 year when well tolerated or until disease progression occurred.

Dose adjustments and treatment modifications

In patients with moderate renal impairment (creatinine clearance 20–39 ml/min), topotecan dosage was reduced to 0.75 mg/m^2 based on the topotecan dose adjustments recommended by Armstrong et al. [22]. No dose adjustments were planned on the basis of hepatic function or age. In all patients, the dose of topotecan was reduced by 0.25 mg/m^2 for subsequent courses for patients who experienced severe neutropenia. During thalidomide treatment, thalidomide dose reductions of 25% were implemented for grade 2 somnolence. The dose was reduced by a further 25% if symptoms did not improve within 48 h. Patients whose somnolence did not improve after the second dose reduction were taken off study.

Patients were taken off study for any drug-related National Cancer Institute Common Toxicity Criteria (NCI-CTC) grade 4 toxicities, any toxicity still unacceptable to the patient despite dose reductions, ALT or AST level $> 2 \times$ ULN, or serum creatinine $> 2 \times$ ULN. Patients were also taken off study if they experienced rapid disease progression or an increase in blast count $> 30\%$ in either the peripheral blood or bone marrow aspirate. Patients could also refuse therapy at any time.

Patient assessments

All patients who received any study medication were evaluable for safety. Adverse events were monitored continuously during treatment and were graded according to NCI-CTC. Complete blood counts (CBC) were monitored at baseline and weekly throughout treatment. During treatment with topotecan, a Chem18 panel was performed before each cycle. The safety and tolerability of the doublet were evaluated by changes in hematologic (i.e. CBC) and other clinical laboratory assessments (i.e. Chem18 panel and other serum biochemical analyses performed at the discretion of the investigator), by physical examination, and by the frequency and severity of adverse events. Serious adverse events included all events of NCI-CTC grade ≥ 3 (non-hematologic) or grade ≥ 4 hematologic toxicities.

Patients who completed three or more cycles of topotecan were evaluable for response. Bone marrow and peripheral blood evaluations were performed at baseline, after three cycles of topotecan (9 weeks) and at 6 months (bone marrow biopsy at 6 months was optional). Bone marrow biopsy samples were assessed for cell morphology, apoptotic index, cytokine levels, and proliferation using established techniques [23,24]. Response was evaluated using a modification of the IWG criteria. Complete response was defined as restoration of normal hematopoiesis and normal peripheral blood counts. Hematologic improvement (HI) was based on erythrocyte improvement only and was defined as the development of transfusion-independence in patients who had transfusion-dependent anemia at baseline. Partial response (PR) was defined as any sustained (≥ 4 weeks, instead of the 2 months required for IWG criteria) benefit in any of the following: $\geq 50\%$ decrease in required number of monthly packed red blood cell transfusions; hemoglobin increase by ≥ 1.0 g/dl; $\geq 30\,000/\mu$l increase in platelet count in patients with baseline measurements $\geq 100\,000/\mu$L; increase in granulocyte count of ≥ 500 cells/μl in patients with baseline leukocyte counts < 3500 cells/μl; $\geq 50\%$ decrease in bone marrow blasts; disappearance of dysplastic features and restoration of normal morphology in one or more cell line in biopsy samples; disappearance of one or more cytogenetic abnormality.

Statistical analysis

Summary and descriptive statistics were used to evaluate demographic and baseline disease characteristics, adverse events, and response data. Disease characteristics were classified using the French–American–British leukemia classification system for MDS [25]. Power calculations were based on the two-sided z-test of the response rate. $P < 0.05$ was considered statistically significant.

Results

Patients

Patient demographics and baseline disease characteristics are shown in Table I. Forty-five patients were enrolled; the median age was 68 years (range 52–79 years). Twenty-seven (60%) patients had RAEB, 12 (27%) patients had RAEB-T, and six (13%) patients had CMML. Karyotype abnormalities were reported

Table I. Patient demographics and baseline disease characteristics.

Parameter	$n = 45$
Median age, years (range)	68 (52–79)
Sex, *n* (%)	
Male	30 (67)
Female	15 (33)
Disease characteristics, *n* (%)*	
RAEB	27 (60)
RAEB-T	12 (27)
CMML	6 (13)
Karyotype, *n* (%)	
Normal	19 (42)
⩾3 abnormalities	12 (27)
Single abnormalities	10 (22)
5q deletion	3 (7)
20q deletion	3 (7)
Trisomy 8	2 (4)
Abnormality–7	2 (4)
Others*	4 (9)
Nature of MDS, *n* (%)	
Primary	42 (93)
Secondary	3 (7)

*Includes one patient each with: two abnormalities, abnormality 21, deletion 7, and inversion 10.
RAEB, Refractory anemia with excess blasts (>5%) in bone marrow; RAEB-T, RAEB with transformation; CMML, chronic myelomonocytic leukemia; MDS, Myelodysplastic syndrome.

in 26 (58%) patients, including 12 (27%) patients who had three or more chromosomal abnormalities. The majority of patients (93%) had primary myelodysplasia.

A total of 139 courses of topotecan were administered, and seven patients went off study before completing three cycles of therapy. Patients received a median of three cycles of topotecan, and less than 25% of the patients received five or more cycles of topotecan. Three patients died before completing topotecan therapy, and these deaths were attributed to complications of disease progression. One patient went off study to recover from hip replacement surgery. The other three patients discontinued therapy because of grade 3 or 4 toxicities including neutropenia (two patients) and syncope (one patient). The remaining 38 patients were evaluable for response.

Twenty-four patients began treatment with thalidomide, and 21 completed therapy per protocol. Of the 24 patients who were treated with thalidomide, three patients discontinued because of thalidomide-related toxicities.

Safety and tolerability

Treatment with topotecan and thalidomide was generally well tolerated (Table II). Although most patients experienced adverse events, most nonhematologic toxicities were mild to moderate in severity,

Table II. Adverse events reported in ⩾ 8% patients ($n = 45$).

	Patients, *n* (%)	
Toxicity	All grades	Grade 3 or 4
Hematologic		
Neutropenia	29 (64)	22 (49)
Thrombocytopenia	23 (51)	14 (31)
Neutropenic fever	4 (9)	3 (7)
Nonhematologic		
Pain	4 (9)	2 (4)
Fatigue	7 (16)	0
Constipation	6 (13)	0
Sweating	5 (11)	0
Rash	4 (9)	0
Weakness	4 (9)	0

and pain was the only grade 3 or 4 nonhematologic toxicity reported. Grade 3 or 4 neutropenia and thrombocytopenia were reported in 22 (49%) and 14 (31%) patients, respectively. The most frequent nonhematologic toxicity was fatigue in seven (16%) patients, and the only grade 3 or 4 adverse events were neutropenic fever in three (17%) patients and pain in two (4%) patients. Grade 1 neuropathy also occurred in three (7%) patients. Three patients died while receiving topotecan (one from pneumonia and two from sepsis), and these events were attributed to complications of disease progression, not to the study medication.

Antitumor response

Thirty-eight patients completed topotecan therapy per protocol and were evaluable for response (Table III). Nine (24%) patients had a PR and showed HI. An additional 13 (34%) patients had stable disease, and 16 (42%) patients experienced disease progression. On an intent-to-treat basis ($n = 45$), the overall response rate was 20%, 29% of patients had stable disease, 36% of patients had disease progression, and 16% of patients were not evaluable.

The disease and response characteristics in the patients who had HI are summarized in Table IV. Of the 27 patients with RAEB, five (19%) patients had bone marrow responses, and three of these patients also had erythroid responses. Of the 12 patients with RAEB-T, three (25%) patients had bone marrow responses and two (17%) had erythroid responses. One of these patients had a trilineage response (in erythrocytes, neutrophils, and platelets). Of the six patients with CMML, one (17%) patient had an erythroid response. Overall, six (16%) of the 38 evaluable patients who initially had refractory anemias became transfusion independent.

The hematologic profiles for the patient who had a trilineage response are shown in Figure 2 [26].

This patient became platelet-transfusion independent within the first month of therapy and red blood cell-transfusion independent within 3 months after starting therapy. Moreover, although this patient had severe neutropenia at study entry, neutrophil levels recovered after 3 months of therapy, and severe neutropenia did not recur for approximately 1 year.

Discussion

Myelodysplastic syndromes encompass a diverse cluster of medical conditions whose treatment remains challenging. Patients enrolled in the present study had MDS with excess blasts or CMML, and topotecan/thalidomide combination therapy produced responses in patients with each of the subtypes. Moreover, many of the common cytogenetic abnormalities associated with high-risk MDS were represented in the patients in the present study. For example, more than half of the patients had abnormal karyotypes, and abnormalities involving single chromosomes were detected in two (4%) patients with single deletions or abnormalities in chromosome 7, three (7%) patients with deletions in chromosome 5, and three (7%) patients with deletions in chromosome 20. An additional two (4%) patients had trisomy of chromosome 8. In comparison, in the general population of MDS patients, 13% have loss of all or part of chromosome 5, 5% have loss of all or part of chromosome 7, 5% have trisomy 8, and $\leqslant 2\%$ have deletions in 17p or 20q or loss of X or Y [2,3]. Therefore, the trial population captured a broad range of MDS phenotypes and cytogenetic abnormalities.

Current therapeutic strategies employ the use of growth factors, blood-product support, cytotoxic chemotherapy, and combined differentiation therapies [27]. Response rates to anticancer therapies are generally poor for patients with high-risk MDS.

Table III. Activity of topotecan/thalidomide therapy.

Response	Evaluable patients, *n* (%) (*n* = 38)
Response	9 (24)
Complete response	0
Partial response (hematologic improvement)	9 (24)
Stable disease	13 (34)
Disease progression	16 (42)

Table IV. Individual patient response data by disease classification.

		Response			
Disease classification	Cytogenetics	Overall	Hematologic	BM blasts	Decrease in cytogenetic abnormality
RAEB	46,XX[20]	PR	HIE	18% to 8%	–
	46,XY,del(5)(q13q31)[20]	PR	HIE	12% to 2%	–
	47,XX, + 8[19]/46,XX[1]	PR	–	Regression to RA 9% to 2%	–
	46,XX,del (5)(q13q33)[16]/46,XX[4]	PR	HIE	Regression to RA 6% to 3%	> 50% decrease in del 5q
	46,XX[16]	PR	–	Regression to RA 17% to 2%	–
RAEB-T	46,XY[20]	PR	HIE	Regression to RA Regression to RAEB	–
	47,XY, + 8,del(20)t(20;21) (p. 10;q10),del(21) t(20;21)(?q13.3;p11.1)[2]/ 48,idem, + Y,del(7)(q11.2q34)[18]	PR	HIN	Regression to RARS	–
	46,XY,del(20)(q11.2q13.3)[3]/ 47,idem,+10[17]	PR	HIE	15% to 3%	
			HIN HI-P	Regression to RA	
CMML	46,XY,del(7)(q11.2)[20]	PR	HIE	-	–

Erythroid response is 100% decrease in transfusion-independent hemoglobin and hemoglobin increase to > 1 g/dl; neutrophil and platelet responses are > 100% increases in cell counts. BM, Bone marrow; RAEB, refractory anemia with excess blasts (> 5%) in bone marrow; PR, partial response; HIE, hematologic improvement-erythroid; RA, refractory anemia; RAEB-T, RAEB with transformation; HIN, hematologic improvement-neutrophils; RARS, RA with ringed sideroblasts; HI-P, hematologic improvement-platelets; CMML, chronic myelomonocytic leukemia.

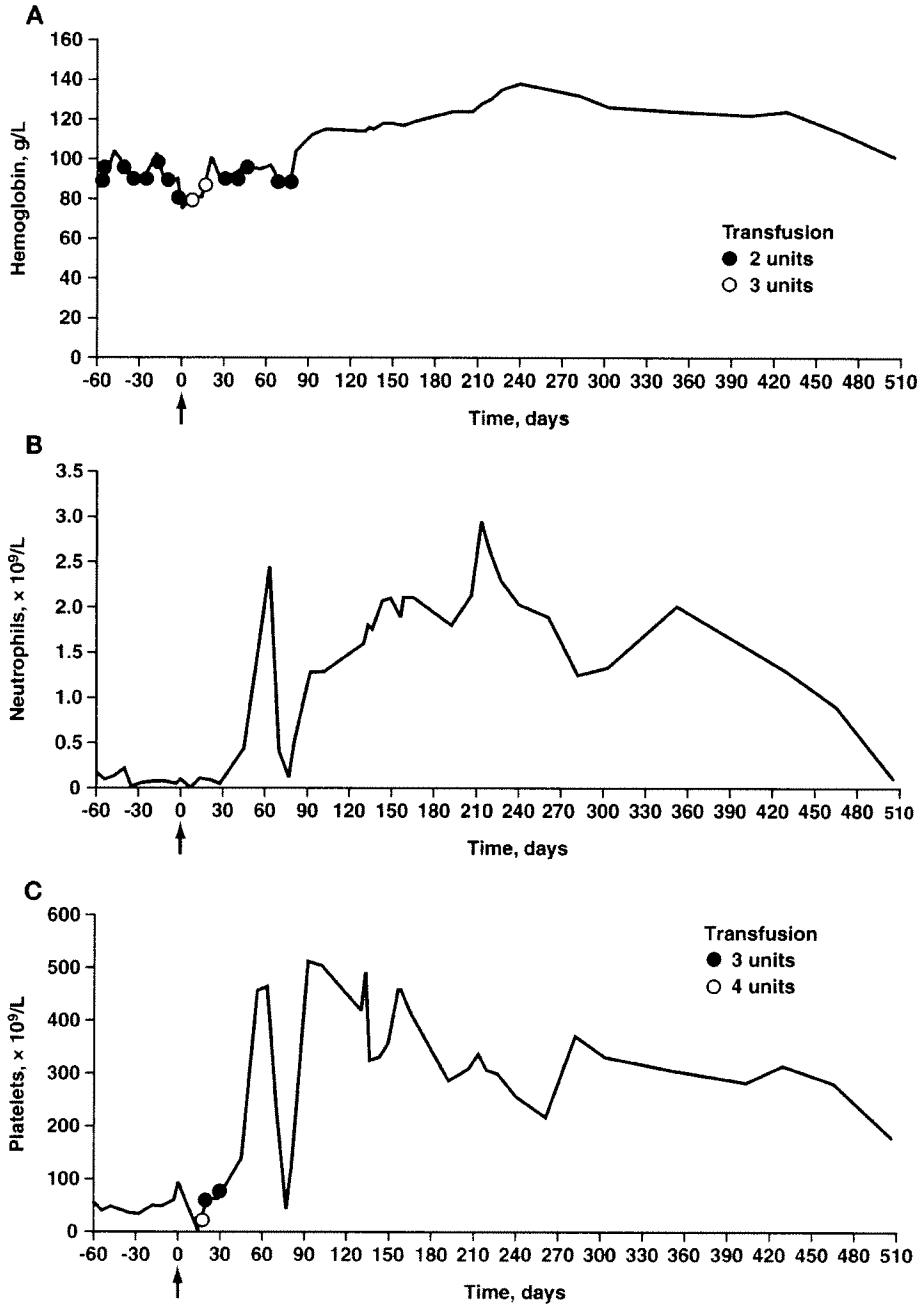

Figure 2. Time course of hematologic parameters in a patient with a good response to therapy. (A) Hemoglobin levels and packed red blood cell transfusion requirements. (B) Absolute neutrophil counts. (C) Platelet counts and platelet transfusion requirements. In all panels, the first day of topotecan therapy is represented as day 0 and indicated with an arrow. Adapted with permission [26].

Alternate treatment options are clearly needed in this setting. Induction and maintenance combination treatment regimens have shown promise, but are of limited efficacy and have not provided any major advances in survival for patients with high-risk MDS. The possible combinations of agents are limited by overlapping toxicities, and increases in dose intensity or treatment duration can result in considerable toxicity [6]. Topotecan has shown promise in early trials in hematologic malignancies [7,8,12–15]. Moreover, topotecan has been successfully used as induction therapy before therapy with carboplatin and etoposide or oral etoposide in patients with small cell lung cancer [28,29], an especially aggressive solid tumor. In that setting, the noncumulative and reversible hematologic toxicity profile of topotecan

allowed hematologic recovery before the initiation of the subsequent therapy. Thalidomide is an attractive agent for use as maintenance therapy following topotecan induction therapy. Although thalidomide has only modest single-agent activity in general [18], it has produced high response rates in some patient subsets [19]. Furthermore, thalidomide has multifaceted antileukemic effects, is safe for long-term therapy in this setting, and can be administered orally [18].

Consistent with observations from trials in other primary malignancies [8], topotecan therapy was well tolerated in the current trial. Although topotecan was associated with neutropenia in patients with solid tumors, neutropenia was generally manageable and reversible. Although two patients discontinued because of high-grade neutropenia and there were three deaths from infections (one from pneumonia and two from sepsis) related to disease progression, the extent to which the hematologic toxicity of topotecan contributed to these deaths is not clear because many patients with high-risk MDS are immunocompromised. Topotecan dose reduction to 1.0 mg/m^2, which is the approved dose level in the relapsed ovarian cancer and relapsed small cell lung cancer settings, may improve hematologic tolerability in future clinical trials in the high-risk MDS setting. However, even at a topotecan dose of 1.25 mg/m^2, nonhematologic toxicity was generally mild, and the only grade 3 or 4 events were pain in two (4%) patients and neutropenic fever in three (7%) patients. This nonhematologic toxicity profile is consistent with that observed in clinical trials of topotecan in solid tumors and was generally manageable, even in heavily pretreated patients.

In the current study, patients who failed to achieve $<5\%$ blasts in their bone marrow after topotecan induction therapy went off study and did not receive subsequent treatment with thalidomide. Consequently, 24 of the original 45 patients received thalidomide, and hematologic parameters in these patients were generally more favourable than in the overall trial population. Thalidomide was generally well tolerated in these patients; three patients discontinued therapy because of associated toxicities. Adverse events during thalidomide therapy included low-grade fatigue, constipation, rash, and mild (grade 1) neuropathy. The incidence of these events in the present trial compared favourably with those reported during thalidomide monotherapy in patients with AML, possibly because of differences in patient populations [18].

This regimen of topotecan induction therapy combined with oral daily thalidomide maintenance therapy had intriguing activity and safety compared to other regimens in the high-risk MDS setting. Although the overall response rate in the 38 evaluable patients was only 24%, an additional 34% of evaluable patients achieved stable disease during therapy. Approximately one-third of the evaluable patients achieved $\geqslant 50\%$ decreases in their bone marrow blasts, and six (16%) patients with refractory anemias achieved transfusion independence. Notably, six of nine patients who had hematologic improvement had relatively favourable baseline cytogenetics. The overall response rate for this regimen is similar to that reported by the Cancer and Leukemia Group B for azacitidine in a recent randomized controlled trial in patients with MDS [30]. However, the Cancer and Leukemia Group B study also enrolled patients with lower-risk MDS (only 66% were of the high-risk groups included in the current study); approximately one half of the patients experienced modified grade 3 or 4 (based on decrease from baseline values) leukopenia, granulocytopenia, and thrombocytopenia, 20% of patients had treatment-related infections, and there was one treatment-related death. Despite predisposing factors, such as the relatively high mean age of the current trial population, very few patients in the current trial experienced any high-grade nonhematologic toxicities.

This present study demonstrated that a regimen combining three 21-day cycles of topotecan 1.25 mg/m^2 by 30-min infusion on days 1–5, followed by 100–300 mg oral daily thalidomide, was generally well tolerated in patients with high-risk MDS. Topotecan/thalidomide therapy demonstrated promising activity in this high-risk MDS trial population, which included a variety of leukemia subclasses and cytogenetic characteristics. However, further studies are needed to determine the optimal dose and schedule of topotecan and thalidomide in patients with long-standing refractory anemias or MDS.

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Exhibit D

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ELSEVIER

LETTER TO THE EDITOR

Phase II study of carboplatin, irinotecan, and thalidomide combination in patients with extensive stage small-cell lung cancer

KEYWORDS
Small-cell lung cancer;
Thalidomide;
Irinotecan;
Extensive stage;
Adverse events;
Anti-angiogenesis

Recently reported phase II and III trials have explored irinotecan in combination with cisplatin or carboplatin in extensive-stage SCLC, revealing it to be well-tolerated and effective [1–3]. Thalidomide has been shown to have activity in numerous malignancies [4,5]. Although the exact anti-tumor mechanism is unknown, thalidomide exhibits both immuno-modulating and anti-angiogenic effects [6]. Based on potentially synergistic mechanisms of action, thalidomide has the potential to enhance the activity of conventional chemotherapy. To examine the efficacy and toxicity of adding thalidomide to conventional cytotoxic agents, carboplatin and irinotecan, we conducted a single-arm phase II study in patients with untreated extensive stage SCLC.

Four cycles of chemotherapy with carboplatin AUC 5 day 1 and irinotecan 50 mg/m^2 i.v. days 1 and 8 were planned every 21 days. Thalidomide was initiated day 1 at 200 mg daily and increased 100 mg weekly to a total dose of 400 mg daily. Hematologic and non-hematologic toxicities were monitored. Overall response was assessed using RECIST criteria.

Six individuals (four males, two females), with a mean age of 65 years (range 53–72), were enrolled (Table 1). Median thalidomide duration was 36 days (range 13–64). No patients were able to tolerate thalidomide beyond ~2 months from study entry. All enrolled patients discontinued thalidomide due to intolerances and associated adverse events. As a result, the study was unable to meet its primary objective of assessing response rate of the triple combination and was stopped by the investigators. Of note, the discontinuation of thalidomide in one patient, due to grade III neutropenia, was not warranted by protocol.

Treatment-related, grade III adverse events included diarrhea, dizziness, drug reaction, neutropenia, and rash. Partial response was identified in two patients. Two grade III thrombotic events (bilateral deep venous thromboses/pulmonary embolism and cerebellar stroke) occurred, but the association to thalidomide administration was uncertain.

This trial proposed the addition of thalidomide to the combination of carboplatin and irinotecan in individuals with extensive stage SCLC. Unfortunately, treatment-related side effects limited not only the ability of patients to titrate thalidomide to the maximum goal but also to continue the medication altogether.

Alternative strategies exploring the potential combination of thalidomide with conventional chemotherapy in SCLC are ongoing. A phase III randomized, double-blind, placebo-controlled multi-center trial, sponsored by the London Lung Cancer Group, plans to investigate the effectiveness of combining carboplatin and etoposide with or without thalidomide in patients with both limited and extensive stage SCLC. Thalidomide is also being investigated as part of a NCI phase II trial for patients with extensive stage SCLC that have responded previously to chemotherapy.

Although the results from this trial suggest that thalidomide in combination with carboplatin and irinotecan is not feasible, the prospects of other anti-angiogenic agents in treating SCLC should not be forsaken. Ongoing studies combining chemotherapy with newer thalidomide-analogues with improved side effect profiles or other vascular-targeting agents offer the possibility of improved tolerability and further assessment of response.

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Table 1 Clinical characteristics and outcomes of enrolled patients

Characteristics	Patients ($n=6$)
Mean Age, years (range)	65 (52–72)
Sex (M:F)	4:2
Performance status	
0–1	4
2	2
Race	
Caucasian	5
African American	1
Presenting symptoms	
Dyspnea	3
Cough	2
Constipation	2
Fatigue	2
Sites involved	
Lung	4
Mediastinal lymph nodes	3
Hilar lymph nodes	2
Outcomes	
Thalidomide discontinuation	
Sustained neutropenia (Grade III)	1
Grade III skin rash	1
Grade III thrombosis	2
Medication intolerance	2
Response, RECIST criteria	
Complete	0
Partial	2
Median thalidomide duration, days (range)	36 (13–64)

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30 January 2006

Exhibit E

DRUG NAME: TOPOTECAN

SYNONYM(S): Topotecan hydrochloride, NSC-609699

COMMON TRADE NAME(S): HYCAMTIN® (notice of compliance,[1] April 1997; patent expires[2] September 2009)

CLASSIFICATION: Topoisomerase I inhibitor, cytotoxic

Special pediatric considerations are noted when applicable, otherwise adult provisions apply.

MECHANISM OF ACTION:

Topotecan is a semisynthetic, water-soluble derivative of camptothecin, which is a cytotoxic alkaloid extracted from plants such as *Camptotheca acuminata*. Topotecan has the same mechanism of action as irinotecan. It inhibits the action of topoisomerase I, an enzyme that produces reversible single-strand breaks in DNA during DNA replication. These single-strand breaks relieve torsional strain and allow DNA replication to proceed. Topotecan binds to the topoisomerase I-DNA complex and prevents religation of the DNA strand, resulting in double strand DNA breakage and cell death.[3] Unlike irinotecan, topotecan is found predominantly in the inactive carboxylate form at neutral pH and it is not a prodrug. As a result, topotecan has different antitumour activities and toxicities from irinotecan.[4] Topotecan is a radiation-sensitizing agent.[5] It is cell cycle phase-specific (S-phase).[6,7]

PHARMACOKINETICS:

Interpatient variability	large interpatient and intrapatient variability[7,8]	
Oral absorption	30-40% absorbed; oral route is being studied in clinical trials.[9,10]	
	time to peak plasma concentration	within 1-2 h[9,10]
Distribution	evenly distributed between blood cells and plasma; extensively distributed into tissues[6]	
	cross blood brain barrier?	CSF to plasma ratio is 29% after a 24-hour infusion and 42% after a 72-hour infusion[6]
	volume of distribution	130 L (reduced by 25% in patients with CrCl of 20-39 mL/min)[3]
	plasma protein binding	35%[3,11]
Metabolism	Undergoes reversible, pH-dependent hydrolysis of the active lactone moiety to the inactive hydroxyacid (carboxylate) form. The lactone form is present at pH ≤ 4 and the hydroxyacid form predominates at physiologic pH. Relatively small amount of topotecan is metabolized by hepatic microsomal enzymes to an active metabolite, *N*-demethyltopotecan.[3,6,12] The clinical significance of this metabolite is not known.[6]	
	active metabolite(s)	lactone form, *N*-demethyltopotecan
	inactive metabolite(s)	hydroxyacid form[3], glucuronides of topotecan and *N*-demethyltopotecan[13]
Excretion	biliary and renal excretion	
	bile	extent of biliary excretion not determined[14]
	urine	20-60% of dose
	terminal half life	2-3 h (increased to 5 h in patients with CrCl of 20-40 mL/min)[3]
	clearance	1030 mL/min (decreased by 33% in patients with CrCl of 40-60 mL/min, by 66% with CrCl 20-40 mL/min); (decreased by 33% with bilirubin of 30-255 μmol/L)[3]
Gender	clearance 24% lower in females but no dosage adjustment required[3,11]	
Elderly	no clinically significant difference in females; no information found on males	

Children	clearance similar to adults when given as a 24-hour continuous infusion
Ethnicity	no information found

Adapted from reference 11 unless specified otherwise. Data pertained to 30 min IV infusion unless specified otherwise.

USES:

Primary uses:
*Ovarian cancer[15-17]

Other uses:
*Lung cancer, small cell[18-20]
Gliomas[21]
Leukemia, acute myelogenous[22,23]
Leukemia, chronic myelomonocytic[24,25]
Lung cancer, non-small cell[26]
Multiple myeloma[27]
Myelodysplastic syndrome[24,25,28]
Neuroblastoma[29]
Pancreatic cancer[30,31]
Retinoblastoma[29]
Rhabdomyosarcoma[29,32]
Sarcoma, Ewing's[29]

*Health Canada Therapeutic Products Programme approved indication

SPECIAL PRECAUTIONS:

Renal dysfunction: Contraindicated in patients with severe renal dysfunction (CrCl < 20 mL/min).[11]

Carcinogenicity: There is some evidence linking therapy with topoisomerase I inhibitors such as topotecan to the development of acute leukemias associated with specific chromosomal translocations. Long-term animal studies have not been done.[3]

Mutagenicity: Mutagenic in mammalian *in vitro* and *in vivo* mutation tests, but not mutagenic in bacterial *in vitro* mutation tests.[3,11]

Fertility: No information found.[3]

Pregnancy: FDA Pregnancy Category D.[3] There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (eg, if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).

Breastfeeding is not recommended due to the potential secretion into breast milk.[3,11]

SIDE EFFECTS:

ORGAN SITE	SIDE EFFECT	ONSET			
	Dose-limiting side effects are in ***bold, italics*** I = immediate (onset in hours to days); E = early (days to weeks); D = delayed (weeks to months); L = late (months to years)				
blood/bone marrow febrile neutropenia	***anemia*** (89%, severe 37%) nadir 15 days, recovery within 7 days[3,16]		E		